



Version with Markings to Show Changes Made

In the Claims:

1. (Thrice Amended) A method of identifying genes essential to growth of a single celled organism comprising:

- (a) preparing a genomic library of the single celled organism;
- (b) providing a plurality of identical grids, each grid comprising a surface on which is immobilized at predefined regions on said surface a plurality of defined materials derived from the genomic library;
- (c) mutagenizing the single celled organism by transfection with (i) a randomly integrated transposon or a similar insertional or transposable element of known sequence or (ii) with a constructed suicide vector;
- (d) growing a test culture comprising the mutagenized single celled organisms and a control culture comprising non-mutagenized single celled organisms under a set of defined conditions;
- (e) harvesting surviving cells from the cultures;
- (f) extracting and isolating DNA from harvested cells of the test culture;
- (g) extracting and isolating RNA or DNA from harvested cells of the control culture;
- (h) generating labeled polynucleotide probes [from the isolated DNA of the test culture using an oligonucleotide primer]wherein an oligonucleotide primer extension reaction directed against (i) the randomly integrated transposon or similar insertional or transposable element of known sequence or (ii) the constructed suicide vector, and the reaction extends into flanking genomic DNA;
- (i) generating labeled polynucleotide probes from the isolated RNA or DNA of the control culture;
- (j) hybridizing the labeled probes generated from the isolated DNA of the test culture to a first identical grid to produce a test hybridization pattern;

(k) hybridizing the labeled probes generated from the isolated RNA or DNA of the control culture to a second identical grid to produce a control hybridization pattern;

(l) comparing the hybridization patterns to identify genes essential for growth in the single celled organism; and

(m) confirming that said identified gene is essential for growth of the single celled organism.

12. (Thrice Amended) A method of identifying genes essential to growth of a single celled organism by identifying conditionally lethal mutant genes, which comprises:

(a) preparing a genomic library of the single celled organism: (i) in an integration vector; or (ii) in an expression vector;

(b) providing a grid comprising a surface on which is immobilized at predefined regions on said surface a plurality of defined materials derived from the genomic library;

(c) mutagenizing the single celled organism by transfection with (i) a randomly integrated transposon or a similar insertional or transposable element of known sequence or (ii) with a constructed suicide vector;

(d) growing the mutagenized single celled organisms under permissive and non-permissive conditions to identify mutagenized single celled organisms containing conditionally lethal mutant genes;

(e) transforming the single celled organism containing said conditionally lethal mutant genes with the genomic library of step (a);

(f) growing the transformed cells under the same non-permissive conditions as step (d) to identify transformed cells in which conditionally lethal mutant genes have been complemented;

(g) harvesting surviving cells;

(h) extracting and isolating DNA from the harvested cells;

(i) generating labeled polynucleotide probes [from the isolated DNA using an oligonucleotide primer] wherein an oligonucleotide primer extension reaction directed against (i) the randomly integrated transposon or similar insertion or transposable element of known sequence or (ii) the constructed suicide vector, and the reaction extends into flanking genomic DNA;

(j) hybridizing the labeled probes generated from the isolated DNA to a grid, whereby such probes that hybridize to the grid identify genes essential for growth of the single celled organism.